Insulin glulisine (glulisine) is a new fast-acting human insulin analogue approved for treatment of diabetes Type 1 and 2. Preclinical investigations indicate mitogenic and metabolic potency similar to human insulin, but distinct β-cell protective properties, possibly related to a preferential effect on the insulin receptor substrate-2. Clinical trials with regular human insulin as a comparator consistently revealed superiority of glulisine with regard to glycemic control, which brought post-prandial glucose excursions closer to physiological levels without increasing the risk of hypoglycemia. Experimental trials showed specific advantages of glulisine in the treatment of obese and pediatric patients, which requires further evaluation. Certainly, glulisine represents a valuable tool for optimizing glycemic control, and thereby for attenuating micro- and macro-vascular complications.

KEYWORDS: β-cells  euglycemic clamp  insulin analogues  insulin-like growth factor  proliferation  obesity  systems biology  zinc

Insulin: physiological action & signaling mechanisms

Metabolism & gene expression
Insulin shifts cellular metabolism towards an anabolic state and is crucial for the systemic control of carbohydrate metabolism (Figure 1) [1]. Insulin increases cellular glucose and amino acid uptake, glycolysis and glucose oxidation, as well as the synthesis of glycogen, proteins, fatty acids and triglycerides. Simultaneously, insulin inhibits catabolic pathways such as gluconeogenesis and glycogenolysis, as well as lipolysis and autophagic proteolysis. Insulin stimulates DNA synthesis and cell proliferation and protects cells from apoptosis – for instance in the setting of liver regeneration [2]. By preventing postprandial excursions of blood glucose, oxidative stress and inflammatory cytokines, insulin provides protection from endothelial dysfunction and vascular disease [3,4]. Insulin promotes complex changes in gene-expression patterns [5,6]. Recent investigations suggest that insulin action in the brain may significantly contribute to the regulation of peripheral glucose and fat metabolism and, in concert with leptin, to hunger and satiety [7,8]. Metabolic insulin effects are antagonized by glucagon, catecholamines and glucocorticoids.

Signal transduction
A complex signaling network activated by insulin binding to its plasma membrane receptor determines the exact insulin effect [9–11] (Figure 1). The insulin receptor (IR) is a receptor tyrosine kinase comprising two extracellular α-subunits and two transmembrane β-subunits. Insulin binding triggers autophosphorylation of the IR-β subunit, which allows recruitment and tyrosine phosphorylation of insulin receptor substrates (IRS), including IRS-1, IRS-2 and the Src homology and collagen protein (Shc). IRS serve as interfaces between the insulin receptor and various signaling pathways leading, for instance, to the activation of PI 3-kinase and protein kinase B (PKB) and mitogen-activated protein (MAP) kinases such as the extracellular signal-regulated kinases Erk-1 and -2 [12]. Cellular internalization of the insulin/IR complex leads to the degradation of insulin initiated by the insulin-degrading enzyme, which may contribute to the termination of insulin signaling [13]. On the other hand, intracellular insulin/IR complexes may even play an active part in signal transduction [14]. As revealed by knockout experiments, insulin-sensitive signal transduction pathways show a high degree of redundancy. For example, genetic deletion of IRS-1 led to the identification of IRS-1-independent signaling via IRS-2 [15–17]. Furthermore, the insulin-sensitive signaling pathway is also addressed by other factors such as insulin-like growth factor (IGF-1), with functional consequences different from those of insulin. Insulin and IGF-1 signals cross-talk with each other by low-affinity binding of insulin to the IGF-1R, and IGF-1 to the IR, respectively, and the engagement of IR/IGF-1R hybrid receptors [18,19]. Among others, the specific outcome of insulin signaling may critically depend on the

Freimut Schliess & Tim Heise
Author for correspondence: Profil Institut für Stoffwechselforschung GmbH, Hellersbergrasse 9, D-41460 Neuss, Germany
Tel.: +49 2131 4018 225; Fax: +49 2131 4018 500; freimut.schliess@profil-research.de
expression profile of cell surface receptors and downstream operating signaling components, the cross-talk between signaling pathways and the spatio–temporal compartmentalization of signaling modules within the cellular matrix.

**Insulin therapy in people with diabetes**

In healthy individuals, nutrient intake provokes a biphasic insulin secretion by the pancreatic β-cells, with maximum blood insulin concentrations achieved within 0.5–1 h, and a return to basal levels within another 2–3 h. The meal-related endogenous insulin production almost perfectly prevents postprandial hyperglycemia, while a low basal insulin secretion accounts for normoglycemia in the post-absorptive state [1,20,21].

Insulin therapy of Type 1 diabetic patients is instituted at the time of diagnosis, and basal-bolus subcutaneous injection regimens or continuous subcutaneous insulin infusion (CSII) aim at mimicking meal-related and basal insulin secretion, which is widely absent in this clinical setting. In Type 2 diabetes, which is characterized by insulin resistance and progressive loss of β-cell function, insulin is often administered on top of an existing treatment with oral antidiabetic drugs (OADs) at later

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**Figure 1. Signaling pathways responsive to insulin, insulin analogues and IGF-1.** The scheme depicts part of the cellular signaling network responsive to insulin, IGF-1 and insulin analogues. Insulin and IGF-1 bind with high affinity to their cognate receptors, and thereby induce distinct, cell-type- and environment-specific signaling patterns. In addition, there is low-affinity binding of insulin (analogues) to the IGF-1 receptor (IGF1-R), and of IGF-1 to the insulin receptor (IR), respectively, and the activation of IR/IGF-1R hybrids induced by ligand binding, autophosphorylation of receptor tyrosine residues generates docking sites for insulin receptor substrates (such as IRS-1, IRS-2 and Shc), which feed signals in different areas of the network. Although signaling pathways can be identified (e.g., the Ras–Raf–MEK–MAPK pathway, the PI 3-kinase–PKB pathway or the intracellular amino acid-dependent signaling via the mammalian target of rapamycin [mTOR]) it should be noted that the activation of specific patterns rather than linear pathways mediates the entire metabolic response to insulin, insulin analogues and IGF-1.

Further information is provided in the text and in [9–14].

Adapted from [10].
stages of the disease, with a stepwise intensification of insulin therapy over the following years [22,23].

**The design of fast-acting insulin analogues**

Subcutaneous insulin injections poorly mimic the physiological pattern of endogenous meal-related and basal insulin secretion. Endogenous, but not subcutaneously administered insulin first passes the liver, which is pivotal for establishment of the portal–peripheral insulin gradient and adequate postprandial glucose control [24]. Furthermore, absorption of regular human insulin (RHI) from a subcutaneous depot into the circulation is delayed by a lag phase of approximately 3–4 h, which accounts for a mismatch between the postprandial rise of blood glucose concentration and the availability of insulin. This increases the incidence of both post-prandial hyperglycemia and late hypoglycemia [25].

**Insulin** is a heterodimetic polypeptide comprising an A chain and a B chain, covalently connected by a disulfide bond between CysB10 and CysA20 (FIGURE 2) [26]. In solution there exists a dynamic equilibrium between monomers, dimers, tetramers, hexamers and possibly higher molecular weight products, which critically depends on insulin concentration, pH and ionic strength [26]. The presence of Zn$^{2+}$ promotes the formation of hexamers, which stabilizes the insulin molecule but delays its absorption following subcutaneous injection [26]. Experimental and theoretical studies established a correlation between the rate of insulin absorption and the ability of hexameric insulin to dissociate into dimers and monomers that can readily permeate the capillary wall [20,25,27]. At a concentration of approximately $10^{-9}$ mol/l in commercially available human insulin preparations, approximately 75% of the insulin molecules assemble to the hexameric form via trimerization of dimers [26]. Dilution of insulin solutions promotes dissociation of the hexameric insulin, and the initial lag phase of insulin absorption reflects the time required for sufficient dilution of subcutaneously deposited insulin hexamers around Zn$^{2+}$, which makes dimeric and monomeric insulin available for absorption.

Fast-acting human insulin analogues were designed with the objective to provide insulin-related drugs that are absorbed faster than RHI, but at the same time ideally match the properties of human insulin regarding receptor binding, post-receptor signaling, mitogenic potency and glucodynamic efficacy. Based on x-ray crystallographic investigations and studies with mutagenated human insulin, amino acid residues were identified that critically contribute to multimerization, Zn$^{2+}$ coordination and binding of insulin to the IR [20,28–32]. This allowed modification of the primary insulin structure to decrease self-assembly to hexamers, and thereby enhance the rate of absorption and consequently improve postprandial glucose disposal after subcutaneous injection. For instance, by replacement of HisB10 by Asp (FIGURE 2), a human insulin analogue was created that cannot hexamerize, even in the presence of Zn$^{2+}$ [31,33,34]. Accordingly, euglycemic clamp experiments with the AspB10 analogue in healthy volunteers revealed a significant faster onset of glucodynamic action as compared with insulin [35,36]. However, in the case of AspB10, a 12-month exposure of rats to high concentrations of the AspB10 analogue increased
The fast-acting insulin analogues currently marketed are summarized in Figure 2. Insulin lispro (lispro) and insulin aspart (aspart) were first approved for the treatment of diabetes mellitus in adults and children by the US FDA in 1996 and 2000, and by the European Medicines Agency (EMEA) in 1996 and 1999, respectively. Both analogues are also available as fixed mixtures of fast- and intermediate-acting (protamine hagedorn (NPH) insulin). Excellent pharmacokinetic and glucodynamic action of lispro and aspart after subcutaneous injection explains the faster absorption and onset of glucose lowering effects in comparison with the obligatory antimicrobial phenolic excipients that occupy hydrophobic interfaces and thereby may confer additional protection of monomeric glulisine from denaturation and fibrillation.

### Structural considerations & formulation of glulisine

Glulisine was derived from human insulin by replacement of Asn\(^{B29}\) by Lys and Lys\(^{B29}\) by Glu (Figure 2), which at physiological pH adds a positive charge in position B3 and replaces a positive charge, at position B29, leading to a formal addition of a negative charge and consequently to a slight decrease of the isoelectric point compared with human insulin. Data on the structural implications of these amino acid replacements were reported in a recent review. Accordingly, the introduction of Lys at position B3 may impair the trimerization of dimers, whereas Glu at position B20 may decrease dimer formation and provide stability to the monomeric glulisine. In contrast to lispro and aspart, Pro\(^{B28}\) remains preserved in glulisine, which then again may support dimerization.

A specific characteristic of the glulisine formulation is the lack of Zn\(^{2+}\) which is not required for glulisine stabilization. Accordingly, glulisine in the marketed formulation exists in the monomeric and the dimeric form only. It was demonstrated that the addition of Zn\(^{2+}\) promotes hexamerization of glulisine, leading to pharmacokinetics and glucodynamic time–action profiles that resemble those of RHI.

Thus, in the case of glulisine the omission of Zn\(^{2+}\) is mandatory to fulfill the characteristics of a fast-acting insulin analogue.

In order to achieve sufficient stability without Zn\(^{2+}\), polysorbate 20 is added to the glulisine formulation, acting as a surfactant that occupies hydrophobic interfaces and thereby may confer additional protection of monomeric glulisine from denaturation and fibrillation.

### Preclinical investigation: glulisine effects on signaling, proliferation, metabolism & β-cell viability

The effects of insulin analogues on signal transduction, metabolism, cell viability and proliferation were examined in vitro, cultured cells (that express different levels of IR and IGF-1 receptors and/or represent relevant insulin target tissues) and in animals in vivo, and usually compared with the respective effects of RHI, IGF-1 and the Asp\(^{B29}\) analogue. Such investigations address safety aspects already at an early stage of drug development and was first approved in 2004 by the FDA and EMEA for the treatment of diabetes mellitus Type 1 and 2 in adults, and in 2008 by the EMEA for the treatment of diabetic children aged 6 years or older.
Insulin glulisine: preclinical hallmarks & clinical efficacy

Signaling via IR & IGF-1R: metabolic & mitogenic potency of insulin analogues

A panel of insulin analogues different from glulisine was initially used to establish a potential relationship between metabolic and mitogenic potency on the one hand, and binding to/dissociation from the IR and the IGF-1R on the other [20,33,42,55,56]. A representative of these studies [55] demonstrated with Chinese Hamster Ovary (CHO) cells that a decreased dissociation from the IR (as estimated in CHO cells overexpressing the human IR) correlates with an increase of both relative metabolic potency (i.e., the analogue concentration normalized to the insulin concentration achieving half-maximal stimulation of glucose uptake or lipogenesis in primary rat adipocytes) and mitogenic potency (i.e., the analogue concentration required to stimulate half-maximal 

\(^{3}H\)thymidine incorporation into the DNA of CHO-K1 cells that do not overexpress the IR) [55]. Remarkably, a disproportionate increase of the mitogenic potency of analogues was observed at Kd values below 40% of the Kd of insulin, which was reflected by an exponential increase in the mitogenic potency:metabolic potency ratio [55]. For instance, the Asp\textsuperscript{10} analogue displayed a relative Kd of approximately 14% of that of insulin, with a metabolic potency of approximately 230% and a mitogenic potency of approximately 650% [55]. The attenuated dissociation of Asp\textsuperscript{10} from the IR was associated with a delayed inactivation of the IR\(\beta\) kinase and a delayed decline of concentration achieving half-maximal stimulation of glucose uptake or lipogenesis in primary rat adipocytes) and mitogenic potency (i.e., the analogue concentration normalized to the insulin concentration required to stimulate half-maximal 

Figure 3. Self-association properties and absorption of regular human insulin and fast-acting insulin analogues. In aqueous solution insulin and its analogues exist in a dynamic equilibrium between monomers, dimers and hexamers. The protracted absorption of RHI from the subcutaneous depot is determined by the dilution-dependent, and therefore time-consuming, dissociation of hexamers, which are stabilized by Zn\textsuperscript{2+} (central sphere). Lispro and aspart in the presence of Zn\textsuperscript{2+} and phenolic excipients form less stable hexamers, which shifts the equilibrium towards the formation of dimers and monomers and thereby accelerates absorption. Based on its structure and marketed formulation glulisine can be stored in the absence of Zn\textsuperscript{2+}, which strongly shifts the equilibrium in favor of dimer and monomer formation. After subcutaneous injection, glulisine becomes immediately absorbed from the depot. RHI: Regular human insulin. Adapted from [51].
IRβ and Shc tyrosine phosphorylation following withdrawal of the analogue from the culture medium, which was suggested to account for the relatively high mitogenicity of the AspB10 analogue [56]. Studies with other cell types established a correlation between IGF-1R binding and the mitogenicity of insulin analogues [56–58].

Remarkably, despite the heterogeneity of receptor binding and dissociation kinetics, the maximal metabolic and proliferative responses to the analogue and the slopes of the respective dose–response curves were almost identical, and not different from those obtained with RHI in cultured cells [55,56]. Furthermore, equimolar amounts of analogues with higher (AspB9) and lower (AspB10) affinity to the insulin receptor and RHI showed an almost equal total glucodynamic activity in vivo in hyperinsulinemic euglycemic clamp experiments [59]. This was explained by a higher/lower rate of IR-mediated clearance of the high-/low-affinity analogues, respectively, which may account for equipotent steady state concentrations in the circulation [20,59].

IR & IGF-1R signaling by glulisine

Studies with cells overexpressing the IR and/or the IGF-1R in vivo investigated glulisine binding and dissociation kinetics, as well as signal transduction downstream of these receptors in view of a potentially increased mitogenicity.

K6 myoblasts derived from rat heart muscle express high levels of the IGF-1R and only small amounts of the IR [60], and were used to study IGF-1R-dependent signaling by glulisine. Glulisine and insulin (each 10−8 mol/l) binding to the K6 myoblasts was comparable and approximately half that of AspB10 [61]. Similarly, the rate of glulisine and insulin internalization was comparable, and approximately 50% that of AspB10. On the other hand, the extent of glulisine degradation was similar to that of AspB10, but only approximately half that of insulin [61]. This indicates that internalization of the analogues was not paralleled by their degradation, and suggests a prolonged intracellular presence of functional IGF-1R/glulisine complexes compared with IGF-1R/insulin.

Glulisine as AspB10 (each 500 nmol/l, 10 min) in the K6 myoblasts increased tyrosine phosphorylation of the IGF-1R approximately 2.5-fold above basal levels, while insulin-induced IGF-1R tyrosine phosphorylation was significantly lower (approximately twofold) [61]. On the other hand, glulisine- and insulin-induced recruitment of Shc to the IGF-1R was comparable, and only approximately a quarter of the Shc recruitment was stimulated by AspB10, which was also true for Shc tyrosine phosphorylation by glulisine, insulin, and AspB10 [61]. Dual phosphorylation of the MAP kinases Erk-1/Erk-2 by glulisine was weaker compared with that induced by insulin and AspB10, while PKB Ser473 and GSK-3α/β Ser21/29 phosphorylation by glulisine, insulin, and AspB10 was almost equal in K6 myoblasts [62]. These findings indicate that the level of receptor occupancy and activation alone may not be sufficient to define the signaling potency of an insulin analogue.

Rat-1 fibroblasts overexpressing the human IR were utilized to study IR-dependent signaling by glulisine. The association kinetics of glulisine (0.0035 nmol/l) were similar to that of insulin, whereas AspB10 displayed a higher affinity to the IR [63]. Consistently, the potency of glulisine and insulin to compete with 125I-labeled AspB10 for IR binding was almost equal and only approximately 10% of that of unlabeled AspB10 [63]. Recording the release of 125I-labeled insulin/analogue from the IR-overexpressing rat-1 fibroblasts revealed similar receptor dissociation kinetics for insulin and glulisine, whereas AspB10 dissociated much more slowly from the IR [63]. Accordingly, the stimulation of maximal IRβ tyrosine phosphorylation (100%) by either glulisine, insulin, or AspB10 (each 1 nmol/l) is followed by a 50% decay in the continued presence of glulisine and insulin, respectively, whereas a pronounced IRβ tyrosine phosphorylation persisted in the presence of AspB10 [63]. Unfortunately, this presentation does not allow comparison of the potencies of glulisine and insulin to increase IRβ tyrosine phosphorylation above basal levels. However, in liver and muscle of random-fed mice, in vivo injection of glulisine or insulin (2 IU) into the inferior vena cava after 10 min increased IRβ tyrosine phosphorylation to comparable levels [64].

A study with human skeletal muscle cells directly compared the affinities of glulisine, insulin, and IGF-1 to both human IR and IGF-1R by measuring the concentrations required for a 50% displacement of specifically bound [125I]insulin and [125I]IGF-1, respectively [65]. Essentially no differences were observed in IR affinities of glulisine and insulin, and both were equally poor for competing IGF-1 binding to the IGF-1R, indicating that affinity of glulisine to the IGF-1R was low and comparable with that of RHI [65].

Mitogenicity of glulisine

Studies performed in different cell culture systems and in animals in vivo suggest a low mitogenic potential of glulisine almost equal to that of human insulin.
Glulisine (16 h) and insulin at the concentration of 500 nmol/l increased the incorporation of 5-bromo-2’-desoxyuridine into the DNA of K6 myoblasts to a similar extent, which was strongly surpassed by AspB10 and IGF-1, respectively [61]. Glulisine and insulin (10–100 nmol/l, 16 h) were also equipotent to stimulate [3H]thymidine incorporation into the DNA of mouse-derived C2C12 myoblasts [64], and glulisine was even less effective than RHI in stimulation of [3H]thymidine incorporation into the DNA of the nonmalignant human breast cell line MCF10 [63]. Furthermore, [3H]thymidine incorporation into the DNA in cultured myotubes derived from nondiabetic and Type 2 diabetic individuals was comparable with glulisine and insulin (each 1–25 nmol/l, 16 h) and only approximately 10% of that observed with IGF-1 in both cell preparations [65]. Similar findings were observed for dual phosphorylation of the MAP kinases Erk-1/Erk-2 in response to glulisine, insulin and IGF-1 [65].

Mammary glands of female rats treated with human insulin or glulisine (20 and 50 IU/kg body weight twice daily over 12 months) showed no difference in the immunoreactivity of the proliferation marker Ki-67 in mammary glands [61]. Unfortunately, no positive control experiment was included in this study.

Glulisine-induced glucose uptake

Uptake of the nonmetabolizable glucose derivative 3-O-methylglucose was used as a surrogate marker for metabolic activity. The initial rise of 3-O-methylglucose uptake by adult rat cardiomyocytes exposed to glulisine, insulin or AspB10 (5 and 500 nmol/l, 10 min) was comparable and in line with the almost equal stimulation of PKB/GSK-3 phosphorylation by insulin and the analogues in these cells [61]. Full dose-response curves recorded in fused and differentiated myotubes derived from nondiabetic and Type 2 diabetic individuals indicated that the sensitivity of 3-O-methylglucose uptake to glulisine and insulin was essentially equal and slightly less than the sensitivity to IGF-1 in these cultures [66]. The findings were reflected at the level of PKB Ser473 phosphorylation, which was similar with regard to sensitivity and maximal response to glulisine and insulin, but higher in response to IGF-1 in both cell preparations [66].

Glulisine signaling via IRS-2: potential impact on β-cell viability

A remarkable finding was that insulin glulisine produced a pronounced tyrosine phosphorylation of IRS-2, but only a marginal, if at all present, tyrosine phosphorylation of IRS-1, whereas a pronounced tyrosine phosphorylation of both IRS-1 and IRS-2 was found in response to insulin and AspB10 [61,67]. This was observed in rat K6 myoblasts, primary human skeletal muscle cells, primary adult rat cardiomyocytes [61] and the clonal rat β-cell line INS-1 [61,67]. In spite of a preferential IRS-2 phosphorylation by glulisine, the metabolic and mitogenic potency of glulisine was similar to human insulin in K6 myoblasts [61]. Preferential IRS-2 tyrosine phosphorylation by glulisine was apparently not related to the relative abundance of IR and IGF-1R.

However, a potential β-cell protective action of glulisine was established in INS-1 cells and related to preferential IRS-2 signaling by this analogue [67]. In these cells tyrosine phosphorylation of IRS-1 by glulisine (500 nmol/l) was virtually absent, whereas insulin, AspB10 (each 500 nmol/l) and IGF-1 (10 nmol/l) produced a pronounced tyrosine phosphorylation of both IRS-1 and IRS-2 [67]. Importantly, insulin glulisine (500 nmol/l) was as potent as IGF-1 (10 nmol/l) to prevent caspase-3 activation and DNA fragmentation as induced by palmitic acid (250 µmol/l, 24 h) or proinflammatory cytokines (4 ng/ml IL-1β plus 10 U/ml IFN-γ, 24 h), whereas RHI and AspB10 were less potent [67]. Recording dose–response curves indicated that glulisine at concentrations of 1–1000 nmol/l was superior to insulin, lispro and aspart in preventing DNA fragmentation in response to palmitic acid and cytokines, respectively [67]. These data suggest that glulisine protects β-cells from fatty acid- and inflammatory cytokine-induced apoptosis.

Studies with a genetic knockout or overexpression of IRS-1/IRS-2 provided substantial evidence for a specific role of IRS-2 in β-cell function. Different from IRS-1 null mice, IRS-2 null mice mimic the phenotype of human Type 2 diabetes, with a decreased β-cell mass and a lack of islet hyperplasia for compensation of insulin resistance [15–17,68]. A recent investigation of mice with a pancreas-specific IRS-2 knockout indicated that pancreatic IRS-2 is not only essential for β-cell mass, but also for adequate insulin secretion in response to glucose [69]. Accordingly, β-cell-specific overexpression of IRS-2 increased β-cell growth, survival and insulin secretion, and prevented diabetes in IRS-2 knock-out mice, obese mice and streptozotocin-treated mice [70,71], while repression of IRS-2 expression by different kinds of stress was associated with increased β-cell apoptosis [72]. From this it seems well conceivable that specific targeting of IRS-2 by glulisine may provide β-cell protection.
According to another scenario, the lack of IRS-1 activation may account for the anti-apoptotic efficacy of glulisine [61,67]. One could hypothesize that IRS-1 in insulin-stimulated β cells, at least in the face of environmental toxins, releases pro-apoptotic signals that are absent in β cells exposed to glulisine. Indeed, a pro-apoptotic potential of insulin became apparent in certain experimental settings [73–75], and there is evidence that IRS-1 could confer such pro-apoptotic signaling. Thus, overexpression of IRS-1 was shown to constitutively activate pro-apoptotic pathways in the liver [76]. In a breast cancer mouse model IRS-1-, but not IRS-2-deficient mammary tumor cells, were highly invasive and resistant to apoptotic treatments [77]. Within this framework, glulisine, by leaving IRS-1 signaling at basal levels, would simply decrease the pro-apoptotic input in β cells, and thereby shift the balance in favor of the IRS-2-mediated survival signals, which may support the β cell to cope with toxins.

Clinical evaluation of glulisine: pharmacokinetics, glucodynamics, safety & tolerability
As insulin glulisine is the third fast-acting insulin analogue on the market, we will briefly describe the clinical benefit of the previously available analogues that have recently been summarized in several meta-analyses. We will then put these data into perspective with those obtained with glulisine in experimental and clinical human trials that were reported as full papers in peer-reviewed journals registered in the PubMed database (Table 1) [204]. Furthermore, where indicated, we discuss some information published in abstract form.

- Fast-acting insulin analogues: meta-analysis of clinical trials
A Cochrane review published in 2006 [48] analyzed 49 randomized, controlled trials (including one with glulisine [78]), with over 8000 participants in total. In terms of % HbA₁c, this study calculated a weighted mean difference of only -0.1% (95% CI: -0.2 to -0.1) in favor of the fast-acting analogues in Type 1 diabetic patients, while no benefit at all was observed in Type 2 diabetes. Although a reliable meta-analysis of hypoglycemic events was compromised by heterogeneous definitions of ‘hypoglycemia’, severe hypoglycemia was stated to occur less often in the analogue group than in the RHI group. Similarly, quality-of-life assessment was not standardized; nevertheless, the frequently used Diabetes Treatment Satisfaction Questionnaire revealed a significant improvement in favor of the analogues. Overall, the Cochrane analysis suggested only a minor clinical benefit of fast-acting insulin analogues in the majority of insulin-treated diabetic patients, and recommended a ‘cautious response to the vigorous promotion of insulin analogues’ until long-term efficacy and safety data would be available.

This analysis was critically commented on in a review published in 2007, which also included randomized, controlled trials, but more selectively than in the Cochrane review. Only studies with an intervention interval of at least 3 months and with a complete change in the insulin regimen to analogues (‘all-analogue’ versus ‘all human insulin’ regimens) were included. Data on postprandial and fasting blood glucose concentrations, weight changes and within- and between-person variability of glucodynamic parameters were analyzed [48]. According to this analysis, which again includes one study with glulisine [78], fast-acting analogues in people with Type 1 diabetes generally decreased postprandial blood glucose concentrations and the incidence of hypoglycemia, compared with RHI. In people with Type 2 diabetes treated with a basal-bolus insulin regimen, the results regarding HbA₁c and hypoglycemia were heterogeneous, but postprandial plasma glucose concentrations were consistently lower with the rapid-acting analogues than with RHI [48]. Furthermore, premixed fast-acting analogues were found to be superior over human insulin mixes in the control of post-prandial blood glucose in Type 2 diabetic people.

Another recent meta-analysis of randomized, controlled trials with an intervention interval of at least 4 weeks (including two studies [79,80] with glulisine) concluded that fast-acting insulin analogues improved both HbA₁c (standardized mean difference: -0.4%, [0.1–0.6%, p = 0.027]) and postprandial blood glucose in comparison with RHI also in people with Type 2 diabetes. These improvements in glucose control were achieved without significant increase in the risk of severe hypoglycemia [47].

- Evaluation of glulisine in healthy nonobese volunteers
The metabolic potency of glulisine in comparison with that of RHI was investigated in a single-center, randomized, open-label, two-way crossover, manual hyperinsulinemic glucose clamp study in 16 healthy male individuals using an intravenous infusion at a rate of 0.8 mU·kg⁻¹·min⁻¹ for 2 h [81]. Overall glucose
### Table 1. Phase III trials evaluating clinical efficacy of glulisine.

<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>Type of Diabetes</th>
<th>Treatment Groups</th>
<th>Number of Randomized Patients, Duration</th>
<th>Mean HbA1c (%) at Baseline</th>
<th>Blood Glucose (mg/dl)</th>
<th>Hypoglycemia</th>
<th>Daily Insulin Dose (IU)</th>
<th>Body Weight (kg)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garg et al. (2005)</td>
<td>Type 1 diabetes, pre-meal GLU vs post-meal GLU vs RHI</td>
<td>860, 12 weeks</td>
<td>7.66</td>
<td>-0.13 (pre-meal GLU vs RHI); -0.15 (pre-meal vs post-meal GLU); n.s. (post-meal GLU vs RHI)</td>
<td>Pre-meal GLU vs RHI, -22.9 n.s. (2 h post-breakfast); -19.9 (2 h post-dinner) Pre-meal GLU vs post-meal GLU, -13.4 (2 h post-breakfast); -11.7 (2 h post-dinner)</td>
<td>Total: -2.31 (pre-meal GLU vs RHI); -2.57 (post-meal GLU vs RHI); -0.26 (pre-meal vs post-meal GLU)</td>
<td>Prandial: -2.63 (pre-meal GLU vs RHI); -2.21 (post-meal GLU vs RHI); -0.41 (pre-meal GLU vs post-meal GLU)</td>
<td>n.s. Total: -0.3 (post-meal GLU vs RHI); -0.3 (post-meal GLU vs pre-meal GLU)</td>
<td>[77]</td>
</tr>
<tr>
<td>Dreyer et al. (2005)</td>
<td>Type 1 diabetes, pre-meal GLU vs pre-meal LIS</td>
<td>683, 26 weeks</td>
<td>7.59</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>Total: -1.87; basal: -1.70</td>
<td>n.s.</td>
<td>[88]</td>
</tr>
<tr>
<td>Dailey et al. (2004)</td>
<td>Type 2 diabetes, GLU vs RHI</td>
<td>878, 26 weeks</td>
<td>7.55</td>
<td>-0.05 (week 12); -0.11 (week 26); -0.11 (end point)</td>
<td>-6.6 (2 h post-breakfast); -10.0 (2 h post-dinner) estimated from FIGURE 2</td>
<td>n.s.</td>
<td>n.s.</td>
<td>[78]</td>
<td></td>
</tr>
<tr>
<td>Rayman et al. (2007)</td>
<td>Type 2 diabetes, GLU vs RHI</td>
<td>892, 26 weeks</td>
<td>7.54</td>
<td>n.s.</td>
<td>Week 12: -10.1 (2 h post-breakfast); -5.0 (2 h, average daily*) End point: -10.1 (2 h post-breakfast); -6.8 (2 h post-dinner); -5.4 (2 h average daily*) estimated from FIGURE 4 Post test meal: -11.0 (1 h); -20.9 (2 h)</td>
<td>Noct: -5.4% Prandial: -1.6 (week 12); n.s. (end point) estimated from FIGURE 3</td>
<td>Not published</td>
<td>[79]</td>
<td></td>
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</tbody>
</table>

*Mean of blood glucose excursions after breakfast, lunch and dinner. The table summarizes data from trials published as a full paper.

GLU: Insulin glulisine; LIS: Insulin lispro; Noct: Nocturnal; n.s.: Not significant; RHI: Regular human insulin.
disposal and the onset of glucodynamic action were similar for intravenously infused glulisine and RHI, as were the shape of the time–concentration profiles [81]. Interpretation of the pharmacokinetic results was hampered by difficulties with the specific assay for glulisine, which produced considerably higher serum concentrations of glulisine than did the human insulin assay for RHI [81]. Distribution and elimination from the systemic circulation of glulisine were comparable to those of RHI [54].

Pharmacokinetics and glucodynamics following subcutaneous injection of glulisine, lispro and RHI (each 0.3 U·kg⁻¹) were compared with a manual hyperinsulinemic, euglycemic clamp in a randomized, double-blind, three-way crossover single-center study in 16 healthy male volunteers, which was published in an abstract form [82]. The data indicated that glulisine and lispro were absorbed significantly faster than RHI. Mean residence time and time to maximum glucose infusion rate were 50% lower for both lispro and glulisine than for RHI. The fast-acting properties of glulisine are preserved independent of the injection site, as demonstrated in a manual glucose clamp study in 16 healthy male volunteers who received glulisine (0.1 IU/kg subcutaneous) into femoral, deltoid or abdominal areas. No significant differences were observed between the sites, although a slightly faster absorption and onset of glucodynamic action was found following abdominal injection [83], as has also been demonstrated for RHI [84].

The effect of mixing glulisine (0.1 IU·kg⁻¹) with NPH (0.2 IU·kg⁻¹) immediately before subcutaneous injection on the time–concentration

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**Figure 4.** Time–concentration and time–action profiles of regular human insulin and glulisine in individuals with Type 1 diabetes. Time–concentration profiles (A & C) and glucose infusion rates (B & D) after subcutaneous administration of glulisine (A & B) and insulin (C & D) were determined in the course of Biostator-based hyperinsulinemic euglycemic clamp experiments. GIR: Glucose infusion rate. Adapted from [87].
profile of glulisine was addressed in a single-dose, randomized, open-label, two-way, cross-over manual hyperinsulminemic euglycemic clamp study in 32 healthy male volunteers published in abstract form [85]. In this study mixing caused a decrease of maximum glulisine concentration by approximately 27% compared with that after separate injections of glulisine and NPH, while a similar total exposure and only a slight decrease of the time period needed to achieve maximum glulisine concentration in the blood was reported [85]. Nevertheless, this study indicates that glulisine should not be mixed with NPH insulin in one syringe, as its fast-acting properties will be blunted. In contrast to the other available fast-acting analogues, lispro and aspart, glulisine is not yet available in a premixed formulation with a free and protamine-retarded component.

Studies in people with Type 1 diabetes

End-organ metabolic effects of glulisine were compared with those of lispro and RHI in a more detailed prospective single-center, double-blind, three-period, cross-over manual hyperinsulminemic euglycemic clamp trial with 18 Type 1 diabetic patients [86]. It was demonstrated that administration of glulisine, lispro and RHI by a continuous intravenous infusion with a stepwise dose increase (0.33, 0.66 and 1.0 mU·kg⁻¹·min⁻¹) had comparable effects on endogenous glucose production and glucose uptake. In addition, effects on the blood concentration of free fatty acids, glycerol and lactate were comparable, indicating that the potency of glulisine is similar to that of lispro and RHI with regard to both blood-glucose-lowering and lipolytic effects.

As in healthy volunteers, glulisine in Type 1 diabetic patients behaved as a fast-acting insulin analogue with pharmacokinetic and glucodynamic time-action profiles comparable with those of lispro [54]. The dose-proportionality of glucose disposal in response to subcutaneous injected glulisine and RHI (each 0.075, 0.15 and 0.3 U/kg) was investigated in a single-center, randomized, Biostator-supported hyperinsulminemic euglycemic clamp study in 18 male Type 1 diabetic patients [87]. As shown in Figure 4, total serum exposure, as well as maximum serum concentrations, were dose-proportional for glulisine and RHI. Furthermore, glulisine exposure in the first 2 h was twice (p < 0.05) that of RHI, and maximum serum concentrations were reached approximately twice (p <0.05) as fast with glulisine as with RHI at all doses.

Accordingly, glulisine-induced glucose disposal in the first 2 h after injection was approximately twice (p < 0.05) as great as that for RHI, whereas overall glucose disposal was almost equal for glulisine and RHI. End-of-action phenomena, such as an increase in blood glucose concentrations at the end of the glucose clamp experiments, were observed earlier with glulisine, confirming a shorter glucodynamic action of the analogue.

Two large Phase III trials in Type 1 diabetic patients investigated glulisine as part of a basal-bolus regimen in combination with a once-daily administration of the basal insulin analogue glargine (Table 1 [78,88]). One of these studies, performed in 683 Type 1 diabetic patients (mean baseline HbA₁c: 7.59%) compared the effects of glulisine and lispro on the basis of percent HbA₁c determinations, self-monitored daily seven-point blood glucose profiles and registration of hypoglycemic episodes [88]. Both insulins resulted in a similar decrease in HbA₁c from baseline (-0.14 and -0.13%, respectively, Figure 5) without significant between-treatment differences, neither in the proportion of patients reaching target HbA₁c values nor in self-monitored glucose concentrations, or in the rate and frequency of symptomatic, severe and nocturnal hypoglycemia. Slight differences were observed concerning the dose of insulin glargine, which was higher in the lispro arm, leading to a very
small increase in the total daily insulin dose compared with glulisine (glulisine: -0.86 IU vs lispro: +1.01 IU, p = 0.0123).

The other Phase III study comparing glulisine with RHI over 12 weeks in 860 people with Type 1 diabetes (mean HbA₁c at baseline: 7.66%) addressed the potential of glulisine with a more flexible timing of injection without losing adequate glycemic control [78]. Glulisine was administered subcutaneously either 0–15 min before or immediately after a meal, whereas RHI was given 30–45 min before a meal, as recommended. 'Pre-meal glulisine' reduced HbA₁c by a significantly greater extent (-0.26%) than RHI (-0.13%, p = 0.02) and the 'postmeal glulisine' (-0.11%, p = 0.006) group, mainly due to significantly lower postprandial blood glucose levels 2 h post-breakfast and 2 h post-dinner (Figure 6). The incidence of hypoglycemia was similar in all treatment arms, as were the number of adverse events. Glycemic control was not significantly different between 'postmeal glulisine' and RHI, but 'postmeal glulisine' led to a small mean decline in body weight (0.3 kg), in contrast to the other treatments that resulted in a gain of 0.3 kg (p = 0.03). Insulin doses, both prandial and total, slightly increased in the RHI group in contrast to a small decrease observed in the two glulisine groups (RHI dose: +1.75 IU, glulisine dose: -0.88 [p = 0.0001] and -0.47 IU [p = 0.0012], respectively).

The potential of postprandial application of insulin glulisine was confirmed in a single-dose, randomized, four-way, complete cross-over study in 20 Type 1 diabetic patients who received a standardized 15-min meal covered by either glulisine (0.15 U·kg⁻¹ per injection immediately pre-meal or 15 min post-meal timed from the start of the meal) or 0.15 U·kg⁻¹ RHI injected 30 min or immediately before meal ingestion [89]. No differences were observed in the blood glucose time–concentration profiles between the '15-min post-meal glulisine', the 'immediately pre-meal RHI' and the '30-min pre-meal RHI' arms. As expected from the glucodynamic properties, with a faster onset of action for glulisine, maximum blood glucose concentrations were lower and reached earlier with the pre-meal injection of glulisine versus the 'immediately pre-meal RHI' group, resulting in lower blood glucose excursions in the first 2 h. Remarkably, the '30-min pre-meal RHI' administration, in contrast to the 'immediate pre-meal glulisine' treatment, led to an early decrease in blood glucose concentrations (Figure 7), indicating a risk of pre-meal hypoglycemia associated with this treatment regimen.

Two additional studies, one clinical and one in vitro, compared glulisine and aspart in patients with CSII. A 12-week, European multicenter study in 59 Type 1 diabetic patients (mean baseline HbA₁c: 6.9%) demonstrated a small (nonsignificant) trend towards fewer catheter occlusions with glulisine, but HbA₁c values, daily insulin doses, blood glucose profiles and adverse event rates were similar [90]. The in vitro simulated CSII study proposed a decreased resistance to fibrillation and a higher rate of soluble high-molecular-weight protein formation with glulisine compared with aspart [91]. In view of the clinical data [90], the relevance of these observations is currently unclear.

### Studies in individuals with Type 2 diabetes

Two large Phase III trials investigated the glycemic control achieved with glulisine or RHI, both in combination with NPH insulin in Type 2 diabetic patients for up to 26 weeks (Table 1) [79,80].

The first study in 878 relatively well-controlled patients (mean HbA₁c: 7.55%) on oral antidiabetic agents (that were continued in this trial) showed improved blood glucose levels at all time points with glulisine, reaching statistical significance 2 h post-breakfast and 2 h post-dinner, resulting in a small, but statistically significant advantage with regard to HbA₁c improvement (-0.46 vs -0.30%, p = 0.0029) [79]. This seems remarkable, in particular as 78% of
the patients mixed glulisine with NPH insulin, which should blunt the advantageous pharmacodynamic properties of glulisine by partial pro-aminization of the analogue [51]. The improved glycemic control by glulisine was neither accompanied by an increased incidence of hypoglycemia (overall, nocturnal and severe), nor due to any increase in insulin or OAD doses. No safety concerns were observed for glulisine; adverse events, laboratory and other safety end points did not show any difference to RHI. Likewise, no statistically significant baseline to end point changes in cross-reactive anti-insulin antibodies were detectable.

The design of the more recently published Phase III trial in Type 2 diabetic patients was similar to that of the first one, but included blood glucose estimations related to ingestion of a standardized test meal at week 26 [80]. This study in 892 patients (mean HbA1c: 7.54% at baseline) did not show any HbA1c differences between glulisine and RHI, so that non inferiority of glulisine was achieved. In addition, the number of patients with at least one episode of nocturnal hypoglycemia from month four to the end of treatment was less frequent with glulisine, but it is unclear if this was a predefined end point or a post-hoc analysis. Blood glucose excursions were significantly lower both in the self-monitored seven-point blood glucose profiles after breakfast and after dinner, as well as after the test meal. No between-treatment differences with regard to frequency and type of adverse events were observed in this study.

Two Phase III studies recently published in abstract form addressed intensification of insulin therapy of Type 2 diabetic patients by addition of glulisine as ‘bolus insulin’ to existing regimens [92,93]. In a randomized, open, multicenter, 26-week study with 318 poorly controlled Type 2 diabetic patients, glulisine was subcutaneously injected either at breakfast or at the meal with greatest glycemic impact (breakfast, lunch or dinner) on top of the already existing ‘OAD plus glargine treatment’ [92]. Glulisine in both arms produced a significant and almost equal HbA1c decrease from baseline to end point, and blood glucose concentrations within each arm were reported to decrease for most pre- and post-meal time points. Whereas the glargine dose remained stable, the glulisine dose increased to a similar extent in both arms. Overall, the study demonstrated that adding glulisine at breakfast is equivalent to adding glulisine at the main meal with no significant difference in the hypoglycemic event rate. Another 52-week study compared a basal-bolus regimen (meal-time glulisine plus once-daily glargine) with a twice-daily injection of pre-mixed insulin (NPH 70%, 30% RHI or aspart) in 310 Type 2 diabetic patients who were poorly controlled with their previous pre-mixed insulins [93]. Compared with the treatment with pre-mixed insulins, the glulisine/glargine regimen provided a significantly improved glycemic control in terms of HbA1c as well as mean daytime and post-prandial blood glucose concentrations without increasing hypoglycemia.

Impact of obesity on the pharmacokinetics & the glucodynamic efficacy of glulisine

The time–concentration profile and glucodynamic action of subcutaneously injected RHI is attenuated and delayed in obese individuals [94–96],
while in case of fast-acting insulin analogues, the influence of obesity may be less pronounced [97–99]. Recent studies compared the pharmacokinetics and glucodynamic time–action profile of glulisine, lispro and RHI in nondiabetic obese individuals [66,100]. In a single dose, randomized double-blind cross-over manual hyperinsulinemic euglycemic clamp study, 18 individuals received subcutaneous injections of either glulisine or RHI (each 0.3 U·kg⁻¹) in predetermined sequences [66]. The study confirmed an accelerated onset of glucose disposal of glulisine and lispro versus RHI, but, interestingly, glulisine showed an even shorter time to achieve 20% of overall glucose disposal, indicating a slightly more rapid onset of glucose disposal than lispro (FIGURE 8). This was confirmed in the pharmacokinetic results, which showed a faster absorption of glulisine than lispro (FIGURE 8). These findings were essentially corroborated in a randomized, single-center, double-blind cross-over study in 80 nondiabetic individuals with a wide range of body mass index (BMI) (from lean [<25 kg/m²] to very obese [>35 kg/m²]). The pharmacokinetics and glucodynamic time–action profiles of lispro and glulisine were compared using two doses (0.2 and 0.4 U·kg⁻¹) in automated hyperinsulinemic euglycemic clamp experiments [100] (FIGURE 9). While total glucose disposal and the time to reach the maximal glucose disposal rate were comparable between the analogues, the onset of glucose disposal as indicated by the time to 10% of overall glucose disposal was slightly, but significantly, earlier for glulisine. Remarkably, the faster onset of glucodynamic action of glulisine was consistently observed with both doses and in all BMI classes, and was confirmed by the pharmacokinetic results that showed a significantly shorter time to reach 10% of total exposure and a significantly higher exposure during the first hour post-dosing for glulisine across BMI ranges.

Obesity-related differences of pharmacokinetics and glucodynamics between glulisine and lispro were not confirmed in an exploratory Biostator-supported hyperinsulinemic euglycemic clamp study in people with Type 2 diabetes published in abstract form [101], which may be, at least partly, due to the incomplete block design used in this study, which leads to high variability in particular in a heterogeneous population such as Type 2 diabetic individuals. A more recent randomized, open-label, two-arm, cross-over test meal study in 15 individuals with Type 2 diabetes compared the plasma glucose and time–concentration profiles following subcutaneous injection of either lispro or glulisine (0.15 U·kg⁻¹ each) immediately prior to a 500 kcal standard test meal (58% carbohydrate, 20% proteins and 22% fat) which was served as breakfast, lunch,

Figure 8. Pharmacokinetic and glycodynamic profile of glulisine, lispro and regular human insulin in obese nondiabetic individuals. Glulisine (spaced dotted curve), lispro (close dotted curve) and RHI (solid curve), each 0.3 U·kg⁻¹, was injected subcutaneously in the abdominal area. Time–concentration profiles (A) and glucose infusion rates (B) were captured in the course of manual hyperinsulinemic euglycemic clamp experiments. Filled areas under the curves indicate the time to 20% exposure to RHI or the analogues, respectively, and the time to 20% of overall glucose disposal in course of the clamp experiments. The data indicate a less intense onset of glucodynamic activity for lispro compared with glulisine.

GIR: Glucose infusion rate; RHI: Regular human insulin. For further information see [66].
and dinner, respectively (Table 1) [102]. The plasma glucose profiles were virtually identical for lispro and glulisine, even though an additional (post hoc) analysis revealed approximately 12% lower baseline-corrected maximum glucose excursions for glulisine. It was also reported that the mean blood glulisine concentrations measured post-meal were significantly higher than that of RHI. However, an apparently higher exposure to glulisine as compared with lispro was also observed in other trials, and considered to be an artifact due to different cross-reactivities of the analogue-specific antibodies utilized in the radioimmune assays [66,81,100]. Post hoc analysis revealed a significantly faster absorption rate for glulisine versus lispro, and some evidence for a linear relationship between the glulisine:lispro ratio and skin thickness over the three meals was mentioned, but data not shown. Both glulisine and lispro were considered safe and well-tolerated by the patients included in this study [102].

## Pediatric studies

The pharmacokinetics and glucodynamic action of glulisine in children and adolescents with Type 1 diabetes was investigated in a randomized, single-dose (0.15 IU·kg⁻¹), double-blind, cross-over study with ten children (aged 5–11 years) and ten adolescents (aged 12–17 years) with a subcutaneous administration of glulisine or RHI 2 min before intake of a standardized test meal (Table 1) [103]. Maximal blood concentrations and initial exposure (during 1 and 2 h after start of the meal) were higher, and the mean residence

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**Figure 9.** Glucodynamic action of glulisine and lispro in lean, overweight and obese nondiabetic individuals. The indicated doses of glulisine and lispro were injected subcutaneously in lean (BMI <25 kg·m⁻²), overweight (≥25 kg·m⁻², <30 kg·m⁻²), obese (≥30 kg·m⁻², <35 kg·m⁻²) and severely obese (≥35 kg·m⁻²) nondiabetic individuals. GIR were registered in the course of automated hyperinsulinemic euglycemic clamp experiments. BMI: Body mass index; GIR: Glucose infusion rates. For further information see [100].
time was shorter for glulisine, whereas total exposure (during 6 h) was comparable for glulisine and RHI. In the case of glulisine, children and adolescents presented almost superimposable time–concentration profiles, whereas a higher (164% [96% CI: 114–236]) exposure was found for RHI in adolescents versus children. Similarly, the maximum blood RHI concentration was higher in adolescents for unknown reasons. In line with the pharmacokinetic data, postprandial glucose excursions were lower with glulisine than with RHI, and blood glucose tended to increase again toward the end of the 6-h monitoring period. The latter finding reflects the absence of basal insulin and is in line with earlier observations [51,104], which suggested that an adjustment of basal insulin may be required when using a new fast-acting insulin analogue. Glulisine was safe and well tolerated in the Type 1 diabetic children and adolescents [103].

Results of a Phase III study comparing the glucodynamic efficacy and safety of glulisine and lispro as part of a basal-bolus regimen in 572 Type 1 diabetic children and adolescents (aged 4–17 years) was published in abstract form [105]. This randomized, parallel-group study reported similar effects for glulisine and lispro on HbA₁c, even though a slightly higher fraction of patients achieved age-specific HbA₁c targets with glulisine (38.4%) than with lispro (32.0%, p = 0.0251). Interestingly, fasting blood glucose concentrations were lower in the glulisine group. No differences were observed in hypoglycemia rates, nor in the frequency and type of adverse events.

**Conclusion**

Compared with RHI, insulin glulisine displays noninferiority with regard to glycemic control in Type 1 and 2 diabetic individuals. Until today no clinical superiority of glulisine over other fast-acting analogues was demonstrated. Preclinical data and experimental human trials point to some specific characteristics of glulisine.

**Specific signaling & cell-protective properties of glulisine**

Although stimulation of DNA synthesis and glucose uptake was comparable with that of RHI [61,63–66], glulisine displayed distinct signaling properties as it preferentially activated IRS-2 in different cell types including the rat pancreatic β-cell line INS-1 [61,67]. This implies that partly different signaling pathways may be involved in regulation of cell proliferation and glucose uptake by glulisine and RHI, respectively. Furthermore, glulisine, in contrast to lispro, aspart and RHI, increased INS-1 cell protection from apoptotic toxins [67], which may be related to the shift from IRS-1– to IRS-2-dependent signaling. Unfortunately, IRS-1/IRS-2 signaling by lispro and aspart was not investigated in this study and it seems conceivable that IRS-1/IRS-2-independent mechanisms account for antiapoptotic signaling by glulisine as well. Further studies are required to dissect IRS-1/IRS-2-dependent and -independent contributions, and to prove that glulisine indeed offers β-cell protection in diabetic animal models in vivo and in individuals with Type 2 diabetes.

Most of the published preclinical studies with insulin analogues provide little information regarding biological action beyond activation of a limited panel of signaling proteins, stimulation of DNA synthesis and glucose disposal. Glulisine, similar to RHI and other insulin analogues, may affect cell function at multiple levels, such as signal transduction, metabolic pathways, gene expression and stress tolerance. From a system biological point of view these levels represent particular projections of a highly complex cellular response [106]. As a first approach to increase insight into the entire biological activity of insulin analogues, gene-expression profiles (transcriptome and proteome) and post-translational protein modifications (e.g., the phosphoproteome) should be recorded with state-of-the-art technology in human cell lines under standardized experimental conditions. Gene-expression profiling was already applied in the biological characterization of insulin mimetic peptides [107]. Such a global approach will certainly help to estimate specific risks and benefits beyond mitogenicity and glycemic control of individual insulin analogues including glulisine.

**Glulisine in the treatment of Type 1 & Type 2 diabetes mellitus in adults & children**

As revealed by hyperinsulinemic euglycemic clamp studies, glulisine in healthy volunteers as well as in patients with Type 1 and 2 diabetes displays pharmacokinetic and glucodynamic profiles that are typical for a fast-acting insulin analogue. Pharmacokinetic characteristics of glulisine versus RHI are closely related to its faster absorption, and include earlier achievement of maximum blood concentration and a shorter mean residence time. This results in a faster onset and a shorter duration of blood glucose-lowering action, which shifts the main effect on glucose disposal closer to the time point of injection. Thus, as part of a basal-bolus
regimen, compared with RHI, glulisine decreases the mismatch between insulin absorption and the postprandial need for glucodynamic activity, and ensures a post-prandial glucose disposal closer to physiological conditions as observed consistently throughout the clinical studies with Type 1 and 2 diabetic patients. This might be of importance, as several epidemiological studies showed a direct correlation between the extent of postprandial blood glucose excursions, but not of fasting values or HbA1c, and the risk for cardio vascular disease [108–110]. A recent exploratory study with Type 2 diabetic patients and healthy volunteers suggests that the oscillations in blood glucose concentrations might be even more deleterious than elevated mean blood glucose concentrations with regard to oxidative stress and endothelial dysfunction [3]. In this context it is noteworthy that glulisine was more effective than RHI in attenuating 3’-nitrotyrosine and asymmetric dimethylarginine formation, as well as intact pro-insulin secretion in Type 2 diabetic patients following ingestion of a test meal [4]. These observations may imply an improved protection from post-prandial oxidative stress and endothelial dysfunction by the analogue. Again, these findings need to be proven in large-scale clinical trials.

Under the controlled conditions of clinical trials, glulisine as compared with RHI causes only a minor, if any, additional decay in HbA1c values. However, glulisine might offer increased flexibility with regard to injection timing, as glucodynamic control, in terms of HbA1c and postprandial blood glucose excursions, was maintained with glulisine administered shortly before or even after ingestion of a meal, while for RHI a 15–30 min interval between injection and meal intake is recommended. Keeping in mind that many diabetic patients ignore this (inconvenient) recommendation risking suboptimal glucodynamic control [111], glulisine might be able to achieve superior glycemic control compared to RHI under daily life conditions, in particular in diabetic patients with irregular eating habits, such as children, adolescents or the elderly.

**Future perspective**

Currently, no human study is available that compares the marketed fast-acting insulin analogues head to head. This makes a prognostic positioning of individual analogues within the upcoming 5–10 years difficult. There is no doubt regarding advantages of the fast-acting insulin analogues over RHI in terms of glycemic control (postprandial blood glucose excursions), suppression of postprandial oxidative stress and time flexibility of administration [22,45–50]. However, due to the lack of large outcome trials, it still remains unclear if these differences are clinically meaningful – that is, if fast acting analogues attenuate the progression of diabetes and the incidence of micro- and macro-vascular complications better than RHI. Experimental trials comparing glulisine and lispro identified some minor advantages of glulisine, in particular a slightly faster onset of action in obese people [66,100] and a small increase in the fraction of patients that achieved age-specific HbA1c targets plus a decrease of fasting blood glucose concentrations in children with Type 1 diabetes [105]. Preclinical studies point to glulisine-specific action profiles beyond glycemic control (preferential targeting of IRS-2, β-cell protection) [61,67].

From the scientific viewpoint, future positioning of glulisine will certainly depend on whether large clinical trials will confirm an additional benefit of glulisine in glycemic control in obese Type 2 diabetic people and diabetic children. In-depth preclinical analysis should focus on the evaluation of insulin and insulin analogues beyond their effects on cellular glucose uptake and DNA synthesis. Such an open-minded research strategy may unravel a pharmacodynamic diversity of insulin analogues and finally define a panel of insulin-related products with distinct action profiles apart from providing adequate glycemic control. Such a full characterization may uncover options for individually tailored insulin therapy regimens, and also profile the fast-acting analogues in competition with upcoming RHI preparations that accelerate insulin absorption merely by modifying the formulation (e.g., [112]).

At present, glulisine is undoubtedly a valuable additional tool in achieving improved glycemic control in people with Type 1 and 2 diabetes, including children and adolescents. The preclinical data are promising that a full functional evaluation of this analogue may uncover additional properties of potential therapeutic benefit.

**Financial & competing interests disclosure**

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.
Glulisine is absorbed faster than RHI. Therefore, pharmacokinetic hallmarks include shorter time to maximum blood concentration and a shorter mean residence time.

This results in a faster onset and a shorter duration of glucodynamic action, which shifts the bulk of glucose disposal closer to the time point of injection.

Glulisine showed a faster onset of action compared with other fast-acting insulin analogues in healthy people irrespective of body mass index. The relevance of this finding in the treatment of diabetes has not yet been established.

Glulisine achieves good glycemic control in Type 1 diabetic children and adolescents.

Glulisine allows a flexible timing of meal-related injection (including the option of postprandial injections) without marked deteriorations in glycemic control.

Clinical effectiveness

Large interventional trials with glulisine as part of a basal-bolus regimen consistently demonstrate that glulisine displays a higher efficacy in the suppression of postprandial glucose excursions than RHI in people with Type 1 and 2 diabetes. This was related to noninferiority to RHI in terms of HbA1c levels and blood glucose concentrations.

Glulisine achieves good glycemic control in Type 1 diabetic children and adolescents.

Glulisine allows a flexible timing of meal-related injection (including the option of postprandial injections) without marked deteriorations in glycemic control.

Safety & tolerability

No safety and tolerability findings of concern were observed in any of the clinical studies.

First-time use of glulisine as a bolus insulin requires dose adaptation of the basal insulin, as is the case for other fast-acting insulin analogues.

Conclusion

Preclinical evaluation: insulin exerts pleiotropic effects on cell metabolism and gene expression, in addition to controlling carbohydrate metabolism. In order to get a reliable estimation of all the effects of insulin analogues, we recommend head-to-head comparisons under standardized experimental conditions. This may include recording of gene-expression profiles and post-translational modifications in standardized cell lines, which may point to specific risks and benefits of individual analogues that deserve further investigation (such as β-cell protection through glulisine).

Clinical efficacy: Although long-term experience is lacking, glulisine is as safe and tolerable as RHI. Due to its pharmacokinetic hallmarks, glulisine is superior to cope with post-prandial blood glucose excursions in Type 1 and 2 diabetic patients. Specific benefits versus other insulin analogues for the treatment of obese and Type 1 diabetic children and adolescents need to be confirmed.

Glulisine represents a valuable tool for optimizing glycemic control, and thereby for attenuating micro- and macro-vascular complications.
**Insulin glulisine: preclinical hallmarks & clinical efficacy**


**This paper (similar to [15]) describes the non-diabetic phenotype of IRS-1 knockout mice. A milestone for the understanding of redundancies in insulin signaling.**


**Describes the diabetic phenotype of IRS-2 knockout mice and provides early and profound evidence for the involvement of IRS-2 in β-cell protection.**


* A comprehensive review that outlines the development of insulin analogues.


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**Broadly introduces the concept of genetically engineered fast-acting insulin analogues in the treatment of diabetes.**


**Introduces the ‘superactive’ insulin analogue AspB10, a prototypal fast-acting analogue which until today serves as a reference in the preclinical evaluation of insulin analogues.**


**This comprehensive review applies the evidence-based approach to the evaluation of insulin analogues and provides in-depth information about the pharmacokinetic and glucodynamic characterization of insulin (analogue) preparations.**
Drugs Evaluation

Schliess & Heise


69 Provides a head-to-head comparison of regular human insulin, lispro, aspart, glulisine, Aspβ10 and IGF-1 with regard to cell-protective properties. By investigation of rat INS-1 insulinoma cells, this study points to a protection of β-cells by glulisine from fatty acid- and inflammatory cytokine-induced apoptosis.


79 * This Phase III trial (similar to [88]) indicates noninferiority of insulin glulisine with regard to glycemic control in Type 1 diabetic people.

80 * This Phase III trial (similar to [80]) indicates noninferiority of insulin glulisine with regard to glycemic control in Type 2 diabetic people.

81 * This Phase III trial (similar to [81]) indicates noninferiority of insulin glulisine with regard to glycemic control in Type 2 diabetic people.

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